REMARKS

Claims 1-61 are pending in this application. Claims 1-27, 31-48, 50 and 57-61 are withdrawn from consideration. Claims 51-54 have been canceled without prejudice or disclaimer. Claim 28 is amended to better clarify what Applicants regard as the invention. Support for the amendment to claim 28 can be found throughout the specification, but in particular on page 31 lines 19-31; page 32, lines 9-19; page 33, lines 6-19; page 68, lines 25-32 and page 69, lines 1-9. New claim 62 has been added for consideration. Support for claim 62 can be found throughout the specification, but particularly on page 24, lines 15-19 and in previously presented claim 28. Accordingly, if new claim 62 is entered for consideration, claims 28-30, 49, 55-56 and 62 are currently under consideration. No new matter is entered by way of this amendment. Reconsideration of this application is respectfully requested.

Applicants' representatives would like to express their sincere appreciation for the courteous and constructive telephonic interview held with Examiner Nickol on November 8, 2005 as related to the claims under consideration. Examiner Nickol noted that the proposed claim amendments would be considered in light of the reference cited. Accordingly, such amendments have been made and are submitted herewith for the Examiner's consideration.

Rejection under 35 USC 102(b)

The Examiner has maintained the rejection of claims 28-30, 49 and 55-56 under 35 U.S.C. 102(b) as being anticipated by Engleman, *et al* (WO94/02156, Feb. 3, 1994) for the following reasons.

The Examiner's Position

While Applicants previously asserted that Engleman, *et al.* do not teach the methods of the present invention as currently claimed, in particular, the step of demonstrating that the cell is in fact apoptotic, using the methods described in the instant application, the Examiner alleges that while the prior art does not specifically teach the procedures recited, said procedures are not *limited* to the method of claim 28. More specifically, the Examiner alleges that because the claim recites that the apoptotic cells "may" be shown to be apoptotic, the procedures are merely optional, not required.

Secondly, with regards to Applicants' argument that the reference does not specifically teach the "irradiation of the antigen" prior to exposure to the dendritic cells for T cell activation, the Examiner notes that the claims do not require such a limitation.

Thirdly, the Examiner alleges that in contrast to Applicants' argument that "The antigens of Engleman, *et al.* are presented to the dendritic cell in the absence of an apoptotic cell.", Engleman, *et al.* specifically teach (page 20, lines 8-10) that "antigens may be used as purified naturally occurring whole polypeptides, purified recombinant whole polypeptides, whole organisms or cells in "viable or dead forms." Further, as set forth previously (Action, mailed 02-16-2005), the Examiner alleges that Engleman, *et al.* clearly teach the exposure of dendritic cells with tumor cells which have been irradiated (page 19).

With respect to the previously submitted Declaration under 37 C.F.R. §1.132 signed by Dr. Albert, which attests to the use of any irradiation, gamma or ultraviolet, which may result in either death of the cell by necrosis or by apoptosis depending on the doses and times of exposure, the Examiner alleges that these arguments that rely on particular distinguishing features are not found persuasive when those features are not recited in the claims.

In addition, the Examiner alleges that since the claims do not require the standard procedures used to detect apoptosis is recited in claim 28 (i.e., Annexin V staining, propidium iodide staining, etc.), the claims only read on contacting dendritic cells with an "apoptotic cell". Furthermoe, the Examiner alleges that the specification defines an "apoptotic cell" (page 24, line 20) as any cell expressing a native or foreign antigen undergoing apoptosis due to *any* condition, including those which usually are associated with causing "necrosis", and when compared to the teachings of the prior art's "dead cell" and irradiated cells, the Examiner alleges that the broad definition of an apoptotic cell is ambiguous, at best, because it would appear that the method would comprise necrotic cells as well because the claims do not unequivocally distinguish necrotic cells from apoptotic cells.

Applicants' Position Regarding Engleman *et al.*

Applicants respectfully traverse the Examiner's rejection and provide further distinctive amendments and arguments herewith, in support of the patentability of the currently amended claims.

Applicants' Invention as Currently Claimed

The present invention is directed to methods of assessing cytotoxic T lymphocyte activity comprising providing an antigen presenting dendritic cell prepared by contacting the dendritic cell with an apoptotic cell expressing an antigen or an apoptotic cell fragment, bleb, or body containing an antigen, wherein said contacting is for a time sufficient to allow said antigen to be internalized by the dendritic cell, and wherein said apoptotic cell expressing or containing said antigen has been induced in vitro to become apoptotic prior to exposure to the dendritic cell; exposing the antigen presenting dendritic cell to a population of T lymphocytes to be assayed for their ability to exhibit killer cell activity; and assaying the cytotoxic activity of the T lymphocytes exposed to said antigen presenting dendritic cell. The antigens of the invention may be tumor antigens or viral antigens and the means by which apoptosis is induced may be selected from a variety of procedures including, but not limited to, ultraviolet light, gamma irradiation, steroids, serum deprivation, cytokines, or drugs which induce apoptosis. Furthermore, the antigen may be produced recombinantly.

More particularly, claim 28 has been amended to recite that **the apoptotic cells expressing or containing the antigen of interest are induced to become apoptotic prior to exposure to the dendritic cell. Accordingly, if a form of irradiation is used to induce apoptosis of the antigen, for example, a tumor cell, the irradiation of that antigen must be done prior to exposure to the dendritic cell.**

In addition, new claim 62 has been added to note that the induction of apoptosis is a required procedure, not an "optional" procedure. Accordingly, the claim now recites that the apoptotic cell **is shown to be apoptotic** by a procedure selected from the group consisting of Annexin V staining, propidium iodide staining, DNA laddering, and staining with dUTP and terminal transferase (TUNEL staining). Applicants assert that

Engleman *et al.* do not teach or suggest that apoptosis, as demonstrated by one of the above-noted procedures, is a requirement.

Applicants' Position Regarding the Engleman *et al.* Reference

As noted previously and maintained in the present Office Action, the Examiner alleges that Engleman *et al.* teach a method of inducing cytotoxic T lymphocyte activity comprising contacting antigen presenting dendritic cells with a variety of antigen donors including bacterial, parasitic, fungal, viral, and tumor antigens, and that the antigens may be purified, recombinant, or exist as whole organisms or cells in viable or dead form. The Examiner further alleges that the reference teaches exposing antigen presenting dendritic cells to a population of T lymphocytes to be assayed for their ability to exhibit killer cell activity and assaying the cytotoxic activity of the T lymphocytes exposed to the antigen presenting DCs. The Examiner further alleges that although the reference does not specifically teach contacting the dendritic cells with "apoptotic cells", Engleman et al. teach that pulsing DCs includes contact with live or irradiated cells. Furthermore, the Examiner alleges that Applicants teach that one means of inducing apoptosis is by irradiation.

Applicants respectfully traverse the Examiner's rejection and assert that in order for a rejection under 35 U.S.C. 102(b) to be proper, a single reference must teach each and every element of the invention as claimed. Engleman et al. do not teach the methods of the present invention as currently claimed. There are distinct differences between the teachings of Engleman *et al.* and the present application.

More particularly, Applicants have amended claim 28 to better clarify what Applicants believe to be the invention. Claim 28 now contains the following phrase:

"...wherein said contacting is for a time sufficient to allow said antigen to be internalized by the dendritic cell, and **wherein said apoptotic cell expressing or containing said antigen has been induced in vitro to become apoptotic prior to exposure to the dendritic cell,...."**

Applicants reiterate their assertions regarding the Engleman *et al* reference, that is, that the intent of Engleman *et al.* was not to induce apoptosis by irradiation of an antigen prior to exposure of the antigen to dendritic cells for cross-presentation to T cells. Further proof that Engelman *et al.* do not contemplate irradiation for inducting apoptosis is found in the Examples on pages 29- 34 of the reference, in particular, in sections 7 and 7.2, whereby Engleman *et al.* specifically **do not teach or suggest irradiation of the antigens, in particular, keyhole limpet hemocyanin (KLH), sperm whale myoglobin (SWM) and HIV gag peptide antigens, prior to exposure to the dendritic cells for T cell activation**. More importantly, Engleman *et al.* **do not transfer these antigens to dendritic cells by way of an apoptotic cell. The antigens of Engleman *et al.* are presented to the dendritic cell in the absence of an apoptotic cell**. Therefore, Engleman *et al.* **do not teach or suggest inducing or assessing T cell activation by way of an apoptotic cell in the context of a dendritic cell**, as Applicants claim. As shown by Engleman *et al.* on page 25, second paragraph, the system of Engleman *et al.* simply calls for the mixing of T cells plus dendritic cells plus antigen. There is no requirement for inducing apoptosis of the cells containing the antigen (by irradiation or by any other means as described in the instant application) **prior to exposure to the dendritic cell.**

As noted in Applicants' response to the previous Office Action, dated July 22, 2005, it is apparent that Engleman *et al.* did not appreciate the complexity of the methods necessary for optimizing the induction or assessment of cytotoxic T lymphocyte killing activity by delivering antigen to a dendritic cell by way of an apoptotic cell. In fact, it was only through the work of the present inventors that such unexpected findings became apparent. For example, as disclosed in the instant application, dendritic cells are noted in the present application as having the ability to efficiently phagocytose apoptotic cells expressing the desired antigen or apoptotic cell fragments, blebs, or bodies containing antigen, and presenting them in the context of HLA antigens for efficient induction and assessment of T cell responses. Once again, Engleman *et al.* **did not irradiate antigens prior to exposure to a dendritic cell, nor did Engleman *et al.* teach or suggest that the apoptotic cells expressing or containing the antigen are to be induced in vitro to become apoptotic prior to exposure to the dendritic cell.** Engelman *et al.* failed to

recognize that antigen may be delivered to a dendritic cell via phagocytosis of an apoptotic cell expressing or containing the antigen for which a T cell response was desired and could be presented in the context of a dendritic cell to T cells for optimal T cell stimulation. More particularly, Engleman *et al.* did not teach or suggest that the antigen presented to the dendritic cell was to be presented in the context of an apoptotic cell, whereby apoptosis could be induced by several different methods, an example of which was by irradiation (gamma or ultraviolet), **which was done prior to exposure to the dendritic cell.** Applicants reiterate that one cannot contemplate a solution without having an appreciation for the problem.

Applicants again respectfully remind the Examiner that it is a fundamental axiom of the patent law that a reference must enable its alleged teachings in order to serve as a proper reference under 35 U.S.C. §102. Applicants again submit that Engleman *et al* do not enable methods of inducing apoptosis since not only is the type of irradiation not indicated, but there are no specified dosages presented, nor times of exposure. In addition, Engleman et al. **do not teach or suggest** methods of inducing or assessing the induction of a T cell response through use of an apoptotic cell for delivery of the antigen to a dendritic cell, **wherein the apoptotic cell containing the antigen is irradiated prior to exposure to the dendritic cell**. Applicants respectfully point out to the Examiner in the Engleman *et al.* reference on page 25, second paragraph:

> "The DC antigen presentation system involves the culturing of T cells or their subsets with autologous or HLA-matched homologous DC in the presence of any antigen."

Applicants assert that Engleman *et al.* do not teach or suggest irradiation (or any other means of inducing apoptosis) of the antigen or a cell expressing or containing said antigen **prior to exposure to the dendritic cell (DC).**

For example, on page 29 of the Engleman *et al.* reference, in section 7.1.2 on line 30, the authors state:

"For the induction of a CD4+ T cell-mediated proliferative response, KLH, SWM, and tetanus toxoid were added to cultures containing DC and CD4+ T cells."

There is absolutely no teaching or suggestion by Engleman *et al.* for the need to present antigen in the context of an apoptotic cell (whereby apoptosis is induced by irradiation or by other means) whereby such cell containing the antigen is irradiated **prior to exposure to the dendritic cell**.

Applicants, on the other hand, teach methods wherein apoptosis of cells containing or expressing the antigen is induced by several different methods, one of which is by irradiation (gamma or ultraviolet) and is measured using various procedures, including but not limited to, Annexin V staining, propidium iodide staining, DNA laddering, or staining with dUTP and terminal transferase (TUNEL staining). Furthermore, as Applicants have noted previously, the instant application points out specific and relevant differences in necrotic cells as compared to apoptotic cells as related to their use for either antigen presentation or maturation of the dendritic cell. It is Applicants' contention that Engleman *et al.* did not appreciate the difference between use of either an apoptotic cell or a necrotic cell for purposes of antigen presentation for inducing or assessing a T cell response. No one could appreciate these differences in outcome of responses until the time of the present invention.

Based on the foregoing, and in summary, Applicants contend that Engleman *et al.* do not contemplate nor appreciate the need for antigen uptake and transfer to the dendritic cell by way of an apoptotic cell, which is then presented to the T cell in the context of the MHC. Applicants further assert that the Engleman *et al* publication **does not teach or suggest** the preparation and use of antigen containing apoptotic cells for induction or assessment of T cell activity, **whereby the cells containing the antigen are induced to become apoptotic prior to exposure to the dendritic cell (DC).** Nor do Engleman *et al.* teach or suggest how to prepare apoptotic cells containing the antigen for delivery to the dendritic cell. In fact, as noted previously, the only antigens taught by Engleman *et al* are KLH, SWM and HIV antigens, **which are not presented in the context of an apoptotic cell**. Furthermore, Engleman et al. **do not teach or suggest that**

the cells are apoptotic by either visual characterization or by monitoring the formation of "blebs" and vesicles at the plasma membrane, cell shrinkage, pyknosis, and increased endonuclease activity, or by using the various other procedures known to measure apoptosis, including the markers Annexin V, propidium iodide, DNA laddering, or staining with dUTP and terminal transferase (TUNEL staining) as a means of determining whether irradiation, if used, induces apoptosis, as presently taught by the inventors of the present application. In all likelihood, if Engleman *et al.* used irradiation to treat tumor cells prior to use as a vaccine, then they most likely used enough gamma irradiation to kill the cells, or to sterilize the preparation of cells, which was not the intention of the present application. In light of the present claim amendments and without the proper teaching of the type of irradiation, as well as the dose or length of time of exposure of tumor cells in Engleman *et al.*, Applicants maintain that Engleman *et al.* do not teach or suggest the methods of the present invention.

Applicants further assert that the rejection under 35 U.S.C. § 102(b) is improper in that the Engleman et al reference is a non-enabling reference. As stated in In re Donohue, 766 F.2d 531, 533, 226 USPQ 619, 621 (Fed. Cir. 1985):

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it. Accordingly, even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. It is not, however, necessary that an invention disclosed in a publication shall have actually been made in order to satisfy the enablement requirement.

Applicants further assert that the use of apoptotic cells as an entity capable of transferring antigen to dendritic cells for cross-presentation to T cells was unknown prior to Applicants' own work. Engleman *et al.* do not teach or suggest that the tumor cells noted on page 19 of their patent application were apoptotic as shown through use of one of the procedures for measuring apoptosis described above and presently taught by the instant application. In addition, Engleman *et al.* **do not teach or suggest** that the antigen-containing apoptotic cells are made apoptotic by irradiation or any other means as taught by the Applicants of the present invention, **prior to exposure to the dendritic cells.**

As shown in the Engleman *et al.* reference on page 25, paragraph two, Engleman *et al.* teach that **their system involves the culturing of T cells with the dendritic cells in the presence of antigen.** As noted above, and re-emphasized here, Engleman ***et al.* do not teach or suggest that the apoptotic cell containing antigen is made apoptotic by irradiation or other means prior to contact with the dendritic cell. Nor do Engleman et al. teach or suggest that apoptosis, as demonstrated by one of the methods noted and claimed in the instant application, is a requirement.**

It is Applicants' contention that Examiner has tried to reconstruct Applicants' invention using hindsight reconstruction, which is impermissible.

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Fees

A check in the amount of $25.00 is enclosed to cover one additional dependent claim as a small entity. No other fees are believed to be due for the present response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

Conclusion

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections and objections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,

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